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Holtzman et al.

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(54) **ASSAY METHOD FOR ALZHEIMER'S DISEASE**

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Related U.S. Application Data

(63) Continuation of application No. 12/834,271, filed on Jul. 12, 2010, now Pat. No. 8,444,977, which is a continuation of application No. 10/486,908, filed as application No. PCT/US02/26321 on Aug. 16, 2002, now Pat. No. 7,771,722.

(60) Provisional application No. 60/313,221, filed on Aug. 17, 2001, provisional application No. 60/334,987, filed on Oct. 23, 2001, provisional application No. 60/313,224, filed on Aug. 17, 2001.

(51) **Int. Cl.**

G01N 33/577	(2006.01)
C07K 16/18	(2006.01)
A61K 39/395	(2006.01)
G01N 33/68	(2006.01)
A61K 39/00	(2006.01)

(52) **U.S. Cl.**

CPC ***C07K 16/18*** (2013.01); ***G01N 33/6896*** (2013.01); *A61K 2039/505* (2013.01); *C07K 2317/24* (2013.01); *C07K 2317/41* (2013.01); *C07K 2317/56* (2013.01); *C07K 2317/92* (2013.01); *G01N 2800/2821* (2013.01)

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CPC C07K 16/18; C07K 2317/24; C07K 2317/92; C07K 14/4711; G01N 33/6896; G01N 2800/2821; G01N 2333/4709; A61K 2039/505; A61K 39/0007

See application file for complete search history.

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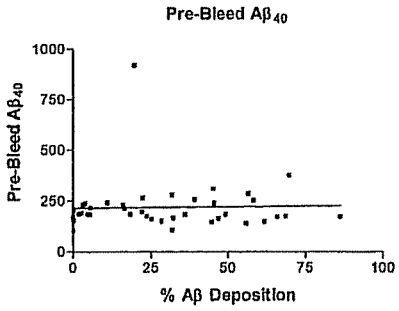
(57) **ABSTRACT**

A diagnostic test for preclinical and clinical Alzheimer's disease is based on plasma levels of $A\beta_{40}$, $A\beta_{42}$, their ratio, or their rate of entry following administration of antibodies that sequester Aβ. Alterations of any of these parameters from control values identifies preclinical or clinical Alzheimer's disease.

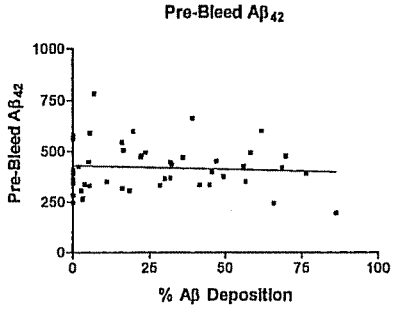
7 Claims, 6 Drawing Sheets

A



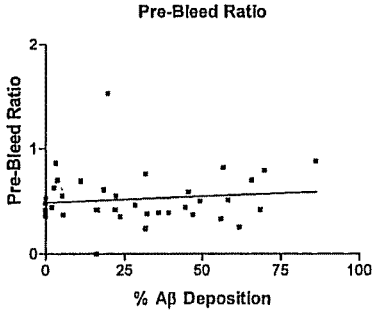
Parameter	PB 40
Number of XY Pairs	42
Pearson r	0.02586
95% confidence interval	-0.2804 to 0.3273
P value (two-tailed)	0.8709
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.0006685

B



Parameter	PB 42
Number of XY Pairs	47
Pearson r	-0.07387
95% confidence interval	-0.3536 to 0.2180
P value (two-tailed)	0.6217
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.005456

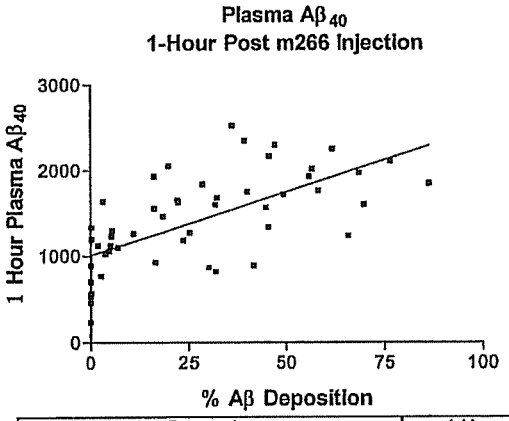
C



Parameter	PB ratio
Number of XY Pairs	40
Pearson r	0.1213
95% confidence interval	-0.1978 to 0.4171
P value (two-tailed)	0.4560
P value summary	ns
Is the correlation significant? (alpha=0.05)	No
R squared	0.01471

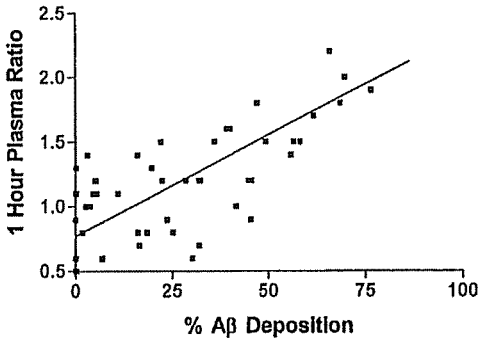
Figure 1

A



Parameter	1-Hour 40
Number of XY Pairs	52
Pearson r	0.6567
95% confidence interval	0.4676 to 0.7884
P value (two-tailed)	P<0.0001
P value summary	***
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.4313

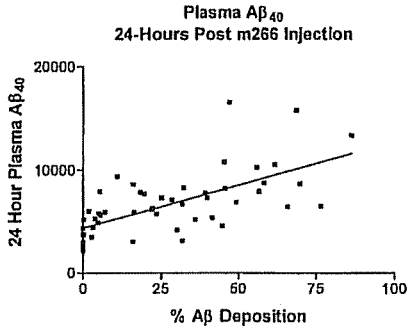
B



Parameter	1-Hour Ratio
Number of XY Pairs	52
Pearson r	0.7565
95% confidence interval	0.6093 to 0.8533
P value (two-tailed)	P<0.0001
P value summary	***
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.5723

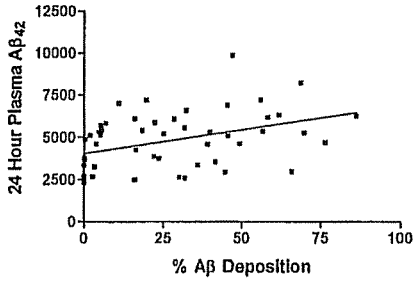
Figure 2

A



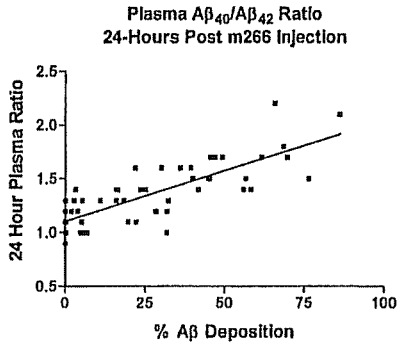
Parameter	24-Hour 40
Number of XY Pairs	52
Pearson r	0.6628
95% confidence interval	0.4759 to 0.7924
P value (two-tailed)	P<0.0001
P value summary	***
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.4393

B



Parameter	24-Hour 42
Number of XY Pairs	52
Pearson r	0.4039
95% confidence interval	0.1471 to 0.6096
P value (two-tailed)	0.0030
P value summary	**
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.1631

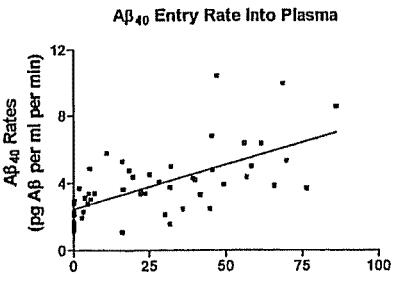
C



Parameter	24-Hour Ratio
Number of XY Pairs	52
Pearson r	0.7987
95% confidence interval	0.6724 to 0.8799
P value (two-tailed)	P<0.0001
P value summary	***
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.6380

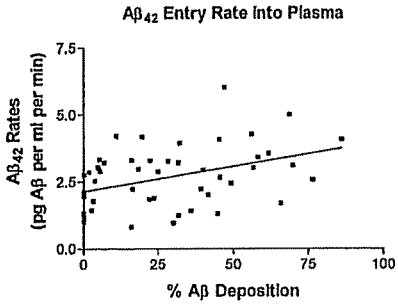
Figure 3

A



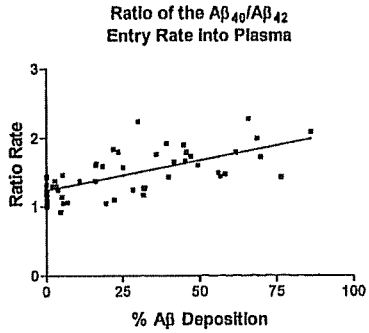
Parameter	40 rate slope
Number of XY Pairs	52
Pearson r	0.6360
95% confidence interval	0.4394 to 0.7745
P value (two-tailed)	P<0.0001
P value summary	***
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.4046

B



Parameter	42 rate slope
Number of XY Pairs	52
Pearson r	0.4062
95% confidence interval	0.1499 to 0.6114
P value (two-tailed)	0.0028
P value summary	**
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.1650

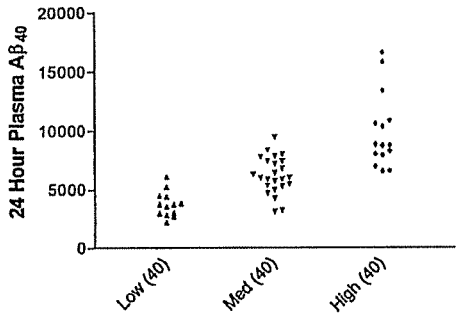
C



Parameter	Ratio Rate
Number of XY Pairs	52
Pearson r	0.6551
95% confidence interval	0.4653 to 0.7873
P value (two-tailed)	P<0.0001
P value summary	***
Is the correlation significant? (alpha=0.05)	Yes
R squared	0.4291

Figure 4

A

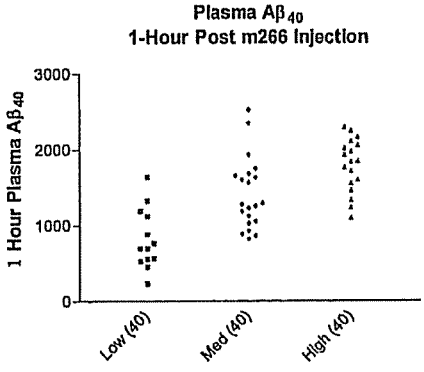


Hippocampus Aβ Coverage

One-way analysis of variance	
P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	3
F	13.88
R squared	0.3616

Tukey's Multiple Comparison Test	P value
Low (42) vs Med (42)	P < 0.01
Low (42) vs High (42)	P < 0.001
Med (42) vs High (42)	P < 0.05

B



Hippocampus Aβ Coverage

P value	P<0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	3
F	20.81
R squared	0.4593

Tukey's Multiple Comparison Test	P value
Low (40) vs Med (40)	P < 0.001
Low (40) vs High (40)	P < 0.001
Med (40) vs High (40)	P < 0.05

Figure 5

Plasma Aβ Correlation's with Alzheimer-Like Pathology in Hippocampus

		Plasma Aβ correlation with Aβ load and fibrillar amyloid						
		Pre-Bleed	5-Min	1-Hour	3-Hour	6-Hour	24-Hour	AUC
Plasma Aβ40:								
Aβ Load:	Pearson r	-0.0158	0.5527	0.5904	0.4310	0.5533	0.5932	0.7056
	P value	0.9209	< 0.0001	< 0.0001	0.0014	< 0.0001	< 0.0001	< 0.0001
Amyloid Load:	Pearson r	0.1535	0.7420	0.6257	0.7053	0.6684	0.7432	0.7624
	P value	0.3378	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Plasma Aβ42:								
Aβ Load:	Pearson r	-0.0614	0.2223	-0.0036	0.1309	0.4551	0.3391	0.5322
	P value	0.6817	0.1207	0.9798	0.3549	0.0008	0.0139	< 0.0001
Amyloid Load:	Pearson r	0.0443	0.4790	0.2321	0.3996	0.4476	0.6062	0.6214
	P value	0.7698	0.0005	0.1013	0.0037	0.0011	< 0.0001	< 0.0001
Aβ40/42 Ratio:								
Aβ Load:	Pearson r	0.0369	0.5223	0.6888	0.4215	0.1754	0.7190	0.6138
	P value	0.8236	< 0.0001	< 0.0001	0.0019	0.2183	< 0.0001	< 0.0001
Amyloid Load:	Pearson r	0.1293	0.4825	0.5047	0.4364	0.2843	0.6029	0.5510
	P value	0.4393	0.0004	0.0002	0.0014	0.0454	< 0.0001	< 0.0001

Figure 6

ASSAY METHOD FOR ALZHEIMER'S DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 12/834,271, filed Jul. 12, 2010, now U.S. Pat. No. 8,444,977, which was filed as a continuation of U.S. patent application Ser. No. 10/486,908, filed Feb. 17, 2004, now U.S. Pat. No. 7,771,722, which was filed as a national stage entry of International Application No. PCT/US02/26321, and claims the benefit of U.S. Pat. Appl. No. 60/313,221, filed Aug. 17, 2001, and U.S. Pat. Appl. No. 60/334,987, filed Oct. 23, 2001, the contents of which are incorporated herein by reference. This application is also related to U.S. Pat. Appl. No. 60/313,224, filed Aug. 17, 2001, the contents of which are incorporated herein by reference.

TECHNICAL FIELD

The invention relates to an assay which permits diagnosis of preclinical and clinical Alzheimer's disease. The test relies on assessing the levels of amyloid beta (Aβ) peptide in plasma following administration of certain anti-Aβ antibodies to a subject.

BACKGROUND ART

A number of symptomologies which result in cognitive deficits, stroke, brain hemorrhage, and general mental debilitation appear to be associated with neuritic and cerebrovascular plaques in the brain containing the amyloid beta peptide (Aβ). Among these conditions are both preclinical and clinical Alzheimer's disease, Down's syndrome, and preclinical and clinical cerebral amyloid angiopathy (CAA). The amyloid plaques are formed from amyloid beta peptides. These peptides circulate in the blood and in the cerebrospinal fluid (CSF). The Aβ peptide in circulating form is composed of 39-43 amino acids (mostly 40 or 42 amino acids) resulting from the cleavage of a common precursor protein, amyloid precursor protein, often designated APP.

Evidence suggests that Aβ can be transported back and forth between brain and the blood (Ghersi-Egea, J-F., et al., *J. Neurochem.* (1996) 67:880-883; Zlokovic, B. V., et al., *Biochem. Biophys. Res. Comm.* (1993) 67:1034-1040; Shibata, M., et al., *J. Clin. Invest.* (2000) 106:1489-1499. Further Aβ in plaques is in an equilibrium with soluble Aβ in the brain and blood (Kawarabayashi, T., et al., *J. Neurosci.* (2001) 21:372-381), DeMattos et al., *Proc. Nat'l. Acad. Sci USA* (2001) 98:8850-8855.

As described in PCT application US00/35681 and U.S. Ser. No. 09/153,130 both incorporated herein by reference, total circulating levels of Aβ peptide in CSF are similar in normal individuals and individuals predisposed to exhibit the symptoms of Alzheimer's. However, $A\beta_{42}$ levels are lower on average in individuals with Alzheimer's disease (Nitsch, R. M., et al., *Ann. Neurol.* (1995) 37:512-518). It is known that $A\beta_{42}$ is more prone to aggregate than is $A\beta_{42}$, and when this happens, adverse consequences such as Aβ deposition in amyloid plaques, conversion of Aβ to toxic forms, nerve cell damage, and behavioral impairment such as dementia ensue (Golde, T. E., et al., *Biochem. Biophys. Acta.* (2000) 1502:172-187).

PCT application PCT/US01/06191 entitled "Humanized Antibodies That Sequester Aβ Peptide" filed 26 Feb. 2001 and incorporated herein by reference describes antibodies which do not appreciably cross the blood-brain barrier and which sequester Aβ peptides circulating in biological fluids. These antibodies are described as useful for preventive and therapeutic treatment of conditions associated with the formation of Aβ-containing diffuse, neuritic, and cerebrovascular plaques in the brain. The application describes administering the antibodies and then measuring circulating levels of Aβ peptide in blood in order to assess the progress of therapy. There is no clear suggestion, however, that the levels of Aβ peptide following administration of the antibodies are diagnostic of the condition itself. The present invention resides in the surprising result that enhanced levels of both $A\beta_{40}$ and $A\beta_{42}$ as well as the $A\beta_{40}/A\beta_{42}$ ratio correlate with the levels of Aβ peptide deposition in the brain when the antibodies are administered to an individual. Thus, measurement of these components in the blood after administration of the antibody provides a simple straightforward diagnostic test for both clinical and preclinical Alzheimer's disease and related neurological disorders.

There are additional relevant publications concerning the behavior of Aβ peptide antibodies. For example, PCT publication W099/27944 published 10 Jun. 1999 describes methods to induce an immune response in order to reduce amyloid deposits. Publication No. W099/60024 published 25 Nov. 1999, describes methods for amyloid removal using anti-amyloid antibodies. Additional PCT publications, including WO00/72880, WO00/72876 and WO00/77178 all describe various activities of anti-Aβ peptide antibodies. Antibodies directed to the N-terminus of this peptide are said to reduce plaques in a transgenic murine model; immunization with the amyloid itself is described as are antibodies designed to catalyze hydrolysis of the peptide.

It has been shown that one pathway for Aβ metabolism is via transport from CNS to the plasma (Zlokovic, B. V., et al., *Proc. Natl. Acad. Sci (USA)* (1996) 93:4229-4234; Ghersi-Egea, J-F., et al., *J. Neurochem.* (1996) 67:880-883). Additionally, it has been shown that Aβ in plasma can cross the blood-brain-barrier and enter the brain (Zlokovic, B. V., et al., *Biochem. Biophys. Res. Comm.* (1993) 67:1034-1040). It has also been shown that administration of certain polyclonal and monoclonal Aβ antibodies decreases Aβ deposition in amyloid plaques in the APP^{V717F} transgenic mouse model of Alzheimer's disease (Bard, F., et al., *Nature Med.* (2000) 6:916-919). This was said to be due to certain anti-Aβ antibodies crossing the blood-brain-barrier and stimulating phagocytosis of amyloid plaques by microglial cells. In Bard's experiments, assays of brain slices ex vivo showed that the presence of added Aβ antibody, along with exogenously added microglia, induced phagocytosis of Aβ, resulting in removal of Aβ deposits.

The levels of both soluble $A\beta_{40}$ and $A\beta_{42}$ in CSF and blood can readily be detected using standardized assays using antibodies directed against epitopes along the Aβ chain. Such assays have been reported, for example, in U.S.

Pat. Nos. 5,766,846; 5,837,672; and 5,593,846. These patents describe the production of murine monoclonal antibodies to the central domain of the Aβ peptide, and these were reported to have epitopes around and including positions 16 and 17. Antibodies directed against the N-terminal region were described as well. Several monoclonal antibodies were asserted to immunoreact with positions 13-28 of the Aβ peptide; these did not bind to a peptide representing positions 17-28, thus, according to the cited patents, establishing that it is this region, including positions 16-17 (the ◇-secretase site) that was the target of these antibodies. Among antibodies known to bind between amino acids 13 and 28 of Aβ are mouse antibodies 266 (m266), 4G8, and 1C2.

DISCLOSURE OF THE INVENTION

It has now been found that antibodies which are useful for performing assays for Aβ peptide, and which are useful in treatment of conditions associated with amyloid plaques in the brain can elicit a response which results in a marked increase in the level of Aβ peptide in the blood and this level can be used as a diagnostic marker for clinical and preclinical Alzheimer's disease. These antibodies, which may or may not be humanized, sequester Aβ peptide from its bound, circulating form in blood and alter clearance of soluble and bound forms of Aβ in central nervous system and plasma. These antibodies, and fragments thereof, specifically bind to an epitope between amino acids 13 and 28 of the Aβ molecule. The CDR of these antibodies can be derived from mouse monoclonal antibody 266 (SEQ ID NO:1 through SEQ ID NO:6). Useful antibodies include antibodies and fragments thereof, wherein the variable regions have sequences comprising the CDR from mouse antibody 266 and specific human framework sequences (SEQ ID NO:7 through SEQ ID NO:10), wherein the antibodies retain approximately the binding properties of the mouse antibody and have in vitro and in vivo properties functionally equivalent to the mouse antibody 266. Especially useful are humanized antibodies and fragments thereof, wherein the light chain is SEQ ID NO:11 and the heavy chain is SEQ ID NO:12.

Thus, in one aspect, the invention is directed to a method to diagnose Alzheimer's disease in a subject at both a clinical and preclinical stage which method comprises administering to said subject an amount of an antibody that sequesters Aβ peptide from its bound, circulating form in blood, and alters clearance of soluble and bound forms of Aβ in the central nervous system in plasma, or which specifically binds an epitope contained within positions 13-28 of Aβ, preferably an antibody having an immunoreactivity equivalent to mouse antibody 266 effective to alter the levels of circulating Aβ peptides in the blood of said subject when said subject is in a clinical or preclinical stage of Alzheimer's disease followed by measuring the level of $A\beta_{40}$, $A\beta_{42}$, or the ratio of $A\beta_{40}/A\beta_{42}$ in the blood of said subject, wherein an enhanced concentration of $A\beta_{40}$, $A\beta_{42}$ and/or $A\beta_{40}/A\beta_{42}$ ratio in said subject identifies said subject as in a preclinical or clinical stage of Alzheimer's disease or cerebral amyloid angiopathy. In other aspects, the invention is directed to kits containing the appropriate materials for conducting the diagnostic method.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1** A, B and C are graphs showing the levels of $A\beta_{40}$ (FIG. **1**A), $A\beta_{42}$ (FIG. **1**B), and $A\beta_{40}/A\beta_{42}$ ratio (FIG. **1**C) in plasma of transgenic mice prior to administration of the antibody m266, and the lack of correlation with brain Aβ deposits.

FIGS. **2** A and B are graphs showing plasma $A\beta_{40}$ (FIG. **2**A) and plasma $A\beta_{40}/A\beta_{42}$ ratio (FIG. **2**B) in transgenic mice one hour after injection of antibody m266, and the significant correlation with brain Aβ deposits.

FIGS. **3** A, B and C are graphs showing the significant correlations of the two Aβ peptides (FIGS. **3**A and **3**B) and their ratio (FIG. **3**C) with Aβ peptide deposition in the brain 24 hours after injection with monoclonal antibody m266.

FIGS. **4** A, B and C are graphs showing the significant correlations of entry rates into the circulation of the two Aβ peptides (FIGS. **4**A and **4**B) and their ratio (FIG. **4**C) and Aβ peptide deposition in transgenic mice.

FIGS. **5** A and B are graphs showing an alternative graphical representation of $A\beta_{40}$ levels in the plasma 24 hours (FIG. **5**A) and 1 hour (FIG. **5**B) after m266 injection correlated with the percentage hippocampus covered by Aβ deposits.

FIG. **6** is a table showing Pearson correlation coefficients (Pearson r) and significance (P value) determined between plasma Aβ values (pre and post injection of m266) and hippocampal Aβ or amyloid load.

MODES OF CARRYING OUT THE INVENTION

The Aβ peptides that circulate in human biological fluids represent a carboxy terminal region of a precursor protein encoded on chromosome 21. It has been reported from the results of in vitro experiments that the Aβ peptide has poor solubility in physiological solutions, since it contains a stretch of hydrophobic amino acids which are a part of the region that anchors its longer precursor to the lipid membranes of cells. It is thus not surprising that circulating Aβ peptide is normally complexed with other moieties that prevent it from aggregating. This has resulted in difficulties in detecting circulating Aβ peptide in biological fluids.

The above-mentioned patent documents (U.S. Pat. Nos. 5,766,846; 5,837,672 and 5,593,846) describe the preparation of antibodies, including a monoclonal antibody, designated clone 266 (m266), which was raised against, and has been shown to bind specifically to, a peptide comprising amino acids 13-28 of the Aβ peptide. Applicants have found that after administering m266 to APP^{V717F} mice, a mouse model of Alzheimer's disease, they can measure levels of Aβ peptides in the circulation that are diagnostic of the levels of amyloid plaques in the brain. Thus, these antibodies are useful not only in conducting assays for circulating Aβ peptides per se, but also for eliciting circulating blood levels which are diagnostic of the amount of amyloid plaque in the brain, and thus useful in identifying individuals in clinical and preclinical stages of Alzheimer's disease. One such antibody, m266, bonds to the mid-region of Aβ peptide.

By "monoclonal antibody that bonds to the mid-region of Aβ peptide" is meant a monoclonal antibody (Mab or Mabs) that binds an amino acid sequence representing an epitope contained between positions 13-28 of Aβ. The entire region need not be targeted. As long as the antibody binds at least an epitope within this region (especially, e.g., including the α-secretase site 16-17 or the site-at which antibody 266 binds), such antibodies are effective in the method of the invention.

By "antibody" is meant a monoclonal antibody per se, or an immunologically effective fragment thereof, such as an F_{ab}, $F_{ab'}$, or $F_{(ab')2}$ fragment thereof. In some contexts, herein, fragments will be mentioned specifically for emphasis; nevertheless, it will be understood that regardless of whether fragments are specified, the term "antibody" includes such fragments as well as single-chain forms. As long as the protein retains the ability specifically to bind its intended target, and in this case, to sequester Aβ peptide from its carrier proteins in blood, it is included within the term "antibody." Also included within the definition "antibody" for example, are single chain forms, generally designated F_v, regions, of antibodies with this specificity. Preferably, but not necessarily, the antibodies useful in the invention are produced recombinantly, as manipulation of the typically murine or other non-human antibodies with the appropriate specificity is required in order to convert them to humanized form. Antibodies may or may not be glycosylated, though glycosylated antibodies are preferred. Antibodies are properly cross-linked via disulfide bonds, as is well-known.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as gamma, mu, alpha, and lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids.

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarily determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2,CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with well known conventions [Kabat "Sequences of Proteins of Immunological Interest" National Institutes of Health, Bethesda, Md., 1987 and 1991; Chothia, et al., *J. Mol. Bio.* (1987) 196:901-917; Chothia, et al., *Nature* (1989) 342:878-883].

As is well understood in the art, monoclonal antibodies can readily be generated with appropriate specificity by standard techniques of immunization of mammals, forming hybridomas from the antibody-producing cells of said mammals or otherwise immortalizing them, and culturing the hybridomas or immortalized cells to assess them for the appropriate specificity. In the present case such antibodies could be generated by immunizing a human, rabbit, rat or mouse, for example, with a peptide representing an epitope encompassing the 13-28 region of the Aβ peptide or an appropriate subregion thereof. Materials for recombinant manipulation can be obtained by retrieving the nucleotide sequences encoding the desired antibody from the hybridoma or other cell that produces it. These nucleotide sequences can then be manipulated to provide them in humanized form, if desired.

It may be desirable to utilize humanized forms of these antibodies in order to elicit the desired circulating levels of the peptides in human subjects. Since the administration is short-term and only for diagnostic purposes, this may not be necessary, but clearly it is preferable to avoid any possibility of an immune response, so the use of humanized forms for this purpose is preferred. Of course, for the performance of the assay of Aβ levels ex vivo (e.g. by ELISA), the murine forms themselves can be used.

By "humanized antibody" is meant an antibody that is composed partially or fully of amino acid sequences derived from a human antibody germline by altering the sequence of an antibody having non-human complementarity determining regions (CDR). The simplest such alteration may consist simply of substituting the constant region of a human antibody for the murine constant region, thus resulting in a human/murine chimera which may have sufficiently low immunogenicity to be acceptable for pharmaceutical use. Preferably, however, the variable region of the antibody and even the CDR is also humanized by techniques that are by now well known in the art. The framework regions of the variable regions are substituted by the corresponding human framework regions leaving the non-human CDR substantially intact, or even replacing the CDR with sequences derived from a human genome. Fully human antibodies are produced in genetically modified mice whose immune systems have been altered to correspond to human immune systems. As mentioned above, it is sufficient for use in the methods of the invention, to employ an immunologically specific fragment of the antibody, including fragments representing single chain forms.

A humanized antibody thus refers to an antibody comprising a human framework, at least one CDR from a non-human antibody, and in which any constant region

present is substantially identical to a human immunoglobulin constant region, i.e., at least about 85-90%, preferably at least 95% identical. Hence, all parts of a humanized antibody, except possibly the CDRs, are substantially identical to corresponding parts of one or more native human immunoglobulin sequences. For example, a humanized immunoglobulin would typically not encompass a chimeric mouse variable region/human constant region antibody.

The design of humanized immunoglobulins may be carried out as follows. When an amino acid falls under the following category, the framework amino acid of a human immunoglobulin to be used (acceptor immunoglobulin) is replaced by a framework amino acid from a CDR-providing non-human immunoglobulin (donor immunoglobulin): (a) the amino acid in the human framework region of the acceptor immunoglobulin is unusual for human immunoglobulin at that position, whereas the corresponding amino acid in the donor immunoglobulin is typical for human immunoglobulin at that position; (b) the position of the amino acid is immediately adjacent to one of the CDRs; or (c) any side chain atom of a framework amino acid is within about 5-6 angstroms (center-to-center) of any atom of a CDR amino acid in a three dimensional immunoglobulin model [Queen, et al., op. cit., and Co, et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:2869]. When each of the amino acid in the human framework region of the acceptor immunoglobulin and a corresponding amino acid in the donor immunoglobulin is unusual for human immunoglobulin at that position, such an amino acid is replaced by an amino acid typical for human immunoglobulin at that position.

A preferred humanized antibody is a humanized form of mouse antibody 266. The CDRs of humanized 266 have the following amino acid sequences:

light chain CDR1:

(SEQ ID NO: 1)

1 5 10 15

Arg Ser Ser Gln Ser Leu Ile Tyr Ser Asp Gly Asn Ala Tyr Leu His

light chain CDR2:

(SEQ ID NO: 2)

1 5

Lys Val Ser Asn Arg Phe Ser

light chain CDR3:

(SEQ ID NO: 3)

1 5

Ser Gln Ser Thr His Val Pro Trp Thr

heavy chain CDR1:

(SEQ ID NO: 4)

1 5

Arg Tyr Ser Met Ser

heavy chain CDR2:

(SEQ ID NO: 5)

1 5 10 15

Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val Lys Gly

and,

heavy chain CDR3:

(SEQ ID NO: 6)

1

Gly Asp Tyr.

A preferred light chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline Vk segments DPK18 and J segment Jkl, with several amino acid substitutions to the consensus amino acids in the same human V subgroup to reduce potential immunogenicity:

(SEQ ID NO: 7)

```
1               5                   10                  15
Asp Xaa Val Met Thr Gln Xaa Pro Leu Ser Leu Pro Val Xaa Xaa

                20                  25                  30
Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Xaa

                35                  40                  45
Tyr Ser Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro

                50                  55                  60
Gly Gln Ser Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe

                65                  70                  75
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp

                80                  85                  90
Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Xaa Gly Val

                95                 100                 105
Tyr Tyr Cys Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Xaa

                110
Gly Thr Xaa Xaa Glu Ile Lys Arg
```

wherein:

Xaa at position 2 is Val or Ile;

Xaa at position 7 is Ser or Thr;

Xaa at position 14 is Thr or Ser;

Xaa at position 15 is Leu or Pro;

Xaa at position 30 is Ile or Val;

Xaa at position 50 is Arg, Gln, or Lys;

Xaa at position 88 is Val or Leu;

Xaa at position 105 is Gln or Gly;

Xaa at position 108 is Lys or Arg; and

Xaa at position 109 is Val or Leu.

A preferred heavy chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segments DP53 and J segment JH4, with several amino acid substitutions to the consensus amino acids in the same human subgroup to reduce potential immunogenicity:

(SEQ ID NO: 8)

```
1               5                   10                  15
Xaa Val Gln Leu Val Glu Xaa Gly Gly Gly Leu Val Gln Pro Gly

                20                  25                  30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

                35                  40                  45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

                50                  55                  60
Xaa Leu Val Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr

                65                  70                  75
Pro Asp Xaa Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa

                80                  85                  90
Xaa Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp

                95                 100                 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly
```

-continued

```
                110
Thr Xaa Val Thr Val Ser Ser
```

wherein:

Xaa at position 1 is Glu or Gln;

Xaa at position 7 is Ser or Leu;

Xaa at position 46 is Glu, Val, Asp, or Ser;

Xaa at position 63 is Thr or Ser;

Xaa at position 75 is Ala, Ser, Val, or Thr;

Xaa at position 76 is Lys or Arg;

Xaa at position 89 is Glu or Asp; and

Xaa at position 107 is Leu or Thr.

A particularly preferred light chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline Vk segments DPK18 and J segment Jkl, with several amino acid substitutions to the consensus amino acids in the same human V subgroup to reduce potential immunogenicity:

(SEQ ID NO: 9)

```
1               5                   10                  15
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu

                20                  25                  30
Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile

                35                  40                  45
Tyr Ser Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro

                50                  55                  60
Gly Gln Ser Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe

                65                  70                  75
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp

                80                  85                  90
Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val

                95                  100                 105
Tyr Tyr Cys Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gln

                110
Gly Thr Lys Val Glu Ile Lys Arg.
```

A particularly preferred heavy chain variable region of a humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segments DP53 and J segment JH4:

(SEQ ID NO: 10)

```
1               5                   10                  15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

                20                  25                  30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

                35                  40                  45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

                50                  55                  60
Glu Leu Val Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr

                65                  70                  75
Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala

                80                  85                  90
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp

                95                  100                 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly

                110
Thr Leu Val Thr Val Ser Ser.
```

A preferred light chain for a humanized antibody of the present invention has the amino acid sequence:

(SEQ ID NO: 11)

```
1               5                   10                  15
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu

                20                  25                  30
Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Ile

                35                  40                  45
Tyr Ser Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro

                50                  55                  60
Gly Gln Ser Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe

                65                  70                  75
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp

                80                  85                  90
Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val

                95                  100                 105
Tyr Tyr Cys Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly Gln

                110                 115                 120
Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val

                125                 130                 135
Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala

                140                 145                 150
Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys

                155                 160                 165
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln

                170                 175                 180
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Her Thr Tyr Ser Leu

                185                 190                 195
Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys

                200                 205                 210
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val

                215
Thr Lys Ser Phe Asn Arg Gly Glu Cys.
```

A preferred heavy chain for a humanized antibody of the present invention has the amino acid sequence:

(SEQ ID NO: 12)

```
1               5                   10                  15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

                20                  25                  30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

                35                  40                  45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

                50                  55                  60
Glu Leu Val Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr

                65                  70                  75
Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala

                80                  85                  90
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp

                95                  100                 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly

                110                 115                 120
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val

                125                 130                 135
```

-continued

```
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala

                140                 145                 150
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr

                155                 160                 165
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe

                170                 175                 180
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val

                185                 190                 195
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys

                200                 205                 210
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val

                215                 220                 225
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro

                230                 235                 240
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro

                245                 250                 255
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr

                260                 265                 270
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe

                275                 280                 285
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys

                290                 295                 300
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val

                305                 310                 315
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys

                320                 325                 330
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr

                335                 340                 345
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr

                350                 355                 360
Leu Pro Pro Her Arg Asp Glu Leu Thr Lys Asn Gin Val Ser Leu

                365                 370                 375
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu

                380                 385                 390
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro

                395                 400                 405
Pro Val Leu Asp Ser Asp Gly Her Phe Phe Leu Tyr Ser Lys Leu

                410                 415                 420
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys

                425                 430                 435
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser

                440
Leu Ser Leu Ser Pro Gly Lys.
```

Other sequences are possible for the light and heavy chains for the humanized antibodies of the present invention and for humanized 266. The immunoglobulins can have two pairs of light chain/heavy chain complexes, at least one chain comprising one or more mouse complementarity determining regions functionally joined to human framework region segments.

Starting at position 56 of the heavy chain variable region, both m266 and humanized 266 contain the sequence Asn-Ser-Thr. This sequence is an example of the Asn-X-Ser/Thr signal for N-linked glycosylation, wherein the Asn is the site of attachment of N-linked glycosyl chains. Both m266 and humanized 266 are extensively glycosylated at this site. Quite unpredictably and advantageously, the affinity of humanized 266 that is deglycosylated in the heavy chain CDR2 for Aβ peptide is markedly higher than that of humanized 266. The heavy chain CDR2 of deglycosylated humanized 266 has the following amino acid sequences:

A preferred deglycosylated humanized antibody is a humanized form of m266, wherein the deglycosylated heavy chain CDR2 is SEQ ID NO:13, wherein:

Xaa at position 7 of SEQ ID NO:13 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr, provided that if Xaa at position 8 is neither Asp nor Pro and Xaa at position 9 is Ser or Thr, then Xaa at position 7 is not Asn;

Xaa at position 8 of SEQ ID NO:13 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr, provided that if Xaa at position 7 is Asn and Xaa at position 9 is Ser or Thr, then Xaa at position 8 is Asp or Pro; and

heavy chain CDR2:

(SEQ ID NO: 13)

```
 1               5                   10                  15
Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val Lys Gly
```

wherein:

Xaa at position 7 is any amino acid, provided that if Xaa at position 8 is neither Asp nor Pro and Xaa at position 9 is Ser or Thr, then Xaa at position 7 is not Asn;

Xaa at position 8 is any amino acid, provided that if Xaa at position 7 is Asn and Xaa at position 9 is Ser or Thr, then Xaa at position 8 is Asp or Pro; and

Xaa at position 9 is any amino acid, provided that if Xaa at position 7 is Asn and Xaa at position 8 is neither Asp nor Pro, then Xaa at position 9 is neither Ser nor Thr;

By "any amino acid" is meant any naturally-occurring amino acid. Preferred naturally-occurring amino acids are Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr.

Xaa at position 9 of SEQ ID NO:13 is selected from the group consisting of Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, and Tyr, provided that if Xaa at position 7 is Asn and Xaa at position 8 is neither Asp nor Pro, then Xaa at position 9 is neither Ser nor Thr.

A preferred heavy chain variable region of a deglycosylated humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segment DP53 and J segment JH4, with several amino acid substitutions to the consensus amino acids in the same human subgroup to reduce potential immunogenicity and wherein the N-glycosylation site in heavy chain CDR2 is modified so that it cannot be N-glycosylated:

(SEQ ID NO: 14)

```
 1               5                  10                  15
Xaa Val Gln Leu Val Glu Xaa Gly Gly Gly Leu Val Gln Pro Gly

            20                  25                  30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

            35                  40                  45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

            50                  55                  60
Xaa Leu Val Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr

            65                  70                  75
Pro Asp Xaa Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa

            80                  85                  90
Xaa Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp

            95                  100                 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly

           110
Thr Xaa Val Thr Val Ser Ser
```

wherein:

Xaa at position 1 is Glu or Gln;

Xaa at position 7 is Ser or Leu;

Xaa at position 46 is Glu, Val, Asp, or Ser;

Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro; and

Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr

Xaa at position 63 is Thr or Ser;

Xaa at position 75 is Ala, Ser, Val, or Thr;

Xaa at position 76 is Lys or Arg;

Xaa at position 89 is Glu or Asp; and

Xaa at position 107 is Leu or Thr.

A particularly preferred heavy chain variable region of a deglycosylated humanized antibody of the present invention has the following amino acid sequence, in which the framework originated from human germline VH segment DP53 and J segment JH4 and wherein the N-glycosylation site in heavy chain CDR2 is modified so that it cannot be N-glycosylated:

(SEQ ID NO: 15)

```
 1               5                  10                  15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

            20                  25                  30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

            35                  40                  45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

            50                  55                  60
Glu Leu Val Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr

            65                  70                  75
Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala

            80                  85                  90
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp

            95                  100                 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly

           110
Thr Leu Val Thr Val Ser Ser.
```

wherein:

Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro; and

Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr.

A preferred heavy chain for a deglycosylated humanized antibody of the present invention, wherein the N-glycosylation site in heavy chain CDR2 is modified so that it cannot be N-glycosylated, has the amino acid sequence:

(SEQ ID NO: 16)

```
  1               5                  10                  15
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly

                 20                  25                  30
Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

                 35                  40                  45
Arg Tyr Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu

                 50                  55                  60
Glu Leu Val Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr

                 65                  70                  75
Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala

                 80                  85                  90
Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp

                 95                 100                 105
Thr Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln Gly

                110                 115                 120
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val

                125                 130                 135
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala

                140                 145                 150
Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr

                155                 160                 165
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe

                170                 175                 180
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val

                185                 190                 195
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys

                200                 205                 210
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val

                215                 220                 225
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro

                230                 235                 240
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro

                245                 250                 255
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr

                260                 265                 270
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe

                275                 280                 285
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys

                290                 295                 300
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val

                305                 310                 315
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys

                320                 325                 330
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr

                335                 340                 345
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr

                350                 355                 360
```

-continued

```
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Her Leu

                365                 370                 375
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu

                380                 385                 390
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro

                395                 400                 405
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu

                410                 415                 420
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys

                425                 430                 435
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser

                440
Leu Her Leu Ser Pro Gly Lys
```

wherein:

Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 59 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro; and

Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr.

Preferred deglycosylated 266 antibodies having the heavy variable region according to SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16 are those wherein:

Xaa at position 56 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 58 is Ser or Thr, then Xaa at position 56 is not Asn;

Xaa at position 57 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr; and

Xaa at position 58 is selected from the group consisting of Ala, Gly, His, Asn, Gln, Ser, and Thr, provided that if Xaa at position 56 is Asn, then Xaa at position 58 is neither Ser nor Thr.

Preferred sequences for CDR2 (positions 56, 57, and 58) of the heavy chain SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16 include those in which only a single amino acid is changed, those in which only two amino acids are changed, or all three are changed. It is preferred to replace Asn at position 56. It is preferred to replace Thr at position 58 with an amino acid other than Ser. It is preferred to not destroy the N-glycosylation site in the CDR2 of the 266 heavy chain by replacing Ser at position 57 with Pro or Asp. Conservative substitutions at one, two, or all three positions are preferred. The most preferred species are those in which Asn at position 56 is replaced with Ser or Thr. Particularly preferred antibodies are those in which Ser or Thr is at position 56, Ser is at position 57, and Thr is at position 58 of SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16.

Especially preferred deglycosylated species are antibodies comprising a light chain of SEQ ID NO:11 and a heavy chain of SEQ ID NO:16, wherein in SEQ ID NO:16, Xaa at position 56 is Ser, Xaa at position 57 is Ser, and Xaa at position 58 is Thr ("N56S"), or wherein in SEQ ID NO:16, Xaa at position 56 is Thr, Xaa at position 57 is Ser, and Xaa at position 58 is Thr ("N56T").

Production of the antibodies useful in the invention typically involves recombinant techniques, as is described in PCT/US01/06191 cited above and incorporated herein by reference.

The antibodies (including immnunologically reactive fragments) are administered to a subject to be evaluated for conditions associated with Aβ deposits such as clinical or preclinical Alzheimer's disease, or clinical or preclinical amyloid angiopathy, using standard administration techniques, preferably peripherally (i.e. not by administration into the central nervous system) by intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration.

The compositions for administration are designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable excipients such as dispersing agents, buffers, surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and the like are used as appropriate. *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton Pa., latest edition, incorporated herein by reference, provides a compendium of formulation techniques as are generally known to practitioners. It may be particularly useful to alter the solubility characteristics of the antibodies of the invention, making them more lipophilic, for example, by encapsulating them in liposomes or by blocking polar groups.

Peripheral systemic delivery by intravenous or intraperitoneal or subcutaneous injection is preferred. Suitable vehicles for such injections are straightforward. In addition, however, administration may also be effected through the mucosal membranes by means of nasal aerosols or suppositories. Suitable formulations for such modes of administration are well known and typically include surfactants that facilitate cross-membrane transfer. Such surfactants are often derived from steroids or are cationic lipids, such as N-[1-(2,3-dioleoyl)propyl]-N,N,N-trimethyl ammonium chloride (DOTMA) or various compounds such as cholesterol hemisuccinate, phosphatidyl glycerols and the like.

The concentration of the humanized antibody in formulations from as low as about 0.1% to as much as 15 or 20% by weight and will be selected primarily based on fluid

volumes, viscosities, and so forth, in accordance with the particular mode of administration selected. Thus, a typical composition for injection could be made up to contain 1 mL sterile buffered water of phosphate buffered saline and 1-1000 mg, preferably 10-100 mg, of the humanized antibody of the present invention. The formulation could be sterile filtered after making the formulation, or otherwise made microbiologically acceptable. A typical composition for intravenous infusion could have volumes between 1-250 mL of fluid, such as sterile Ringer's solution, and 1-100 mg per mL, or more in antibody concentration. Therapeutic agents of the invention can be frozen or lyophilized for storage and reconstituted, in a suitable sterile carrier prior to use. Lyophilization and reconstitution can lead to varying degrees of antibody activity loss (e.g. with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies). Dosages may have to be adjusted to compensate. The pH of the formulation will be selected to balance antibody stability (chemical and physical) and comfort to the patient when administered. Generally, pH between 4 and 8 is tolerated.

Although the foregoing methods appear the most convenient and most appropriate for administration of proteins such as humanized antibodies, by suitable adaptation, other techniques for administration, such as transdermal administration and oral administration may be employed provided proper formulation is designed.

In addition, it may be desirable to employ controlled release formulations using biodegradable films and matrices, or osmotic mini-pumps, or delivery systems based on dextran beads, alginate, or collagen.

In summary, formulations are available for administering the antibodies of the invention and are well-known in the art and may be chosen from a variety of options.

Typical dosage levels can be optimized using standard clinical techniques and will be dependent on the mode of administration.

After administration of the antibody to the subject, blood samples are withdrawn at periodic intervals over the succeeding minutes, hours, or days. Suitable time periods may be as short as a few minutes, 10 minutes, 30 minutes, or 1 hour, several hours, or days may be allowed to elapse before withdrawal of the blood sample. Measurement after less than 3 hours is preferred. If desired, the plasma fraction can be obtained for ease of analysis. Standard analytic techniques for analysis of the $A\beta_{40}$, $A\beta_{42}$ and the ratio thereof are used. These techniques are described, for example, in U.S. Pat. No. 5,766,846. Any suitable technique for analysis, however, can be employed, such as chromatographic separation, Western blotting, ELISA assays, homogenous assays and the like.

The concentration of the $A\beta_{40}$, $A\beta_{42}$, or their ratio is then compared to these values in a control. Typical controls include individuals known to be free of conditions associated with the amyloid plaques, such as teenagers or very young adults and in addition, age-matched cognitively normal controls are obtained by averaging values from the general population. While some elderly age-matched cognitively normal controls have pre-clinical AD, most do not. Thus, the average values from such a population will be useful and critical to obtain. Design of standard controls is a process that is well known to the ordinary practitioner. Individuals who have elevated levels of the stated peptides or of the ratio of $A\beta_{40}$ to $A\beta_{42}$ as compared to the control values are then identified as having a high likelihood of clinical or preclinical conditions associated with the formation of amyloid plaques.

It may be desirable to package the components for carrying out the assay of the invention into convenient kits. Such kits will include containers such as bottles or vials which contain samples of the antibody to be administered as well as the appropriate reagents for carrying out the assay on the withdrawn blood sample. The kit will also contain instructions for conducting the assay and, optionally, charts of control values.

The following examples are intended to illustrate but not to limit the invention.

The examples hereinbelow employ, among others, a murine monoclonal antibody designated "266" which was originally prepared by immunization with a peptide comprised of residues 13-28 of human Aβ peptide. The antibody was confirmed to immunoreact with this peptide, but had previously been reported to not react with the peptide containing only residues 17-28 of human Aβ peptide, or at any other epitopes within the Aβ peptide. The preparation of this antibody is described in U.S. Pat. No. 5,766,846, incorporated herein by reference. As the examples here describe experiments conducted in murine systems, the use of murine monoclonal antibodies is satisfactory. However, in the treatment methods of the invention intended for human use, humanized forms of the antibodies with the immunospecificity corresponding to that of antibody 266 are preferred.

Example 1

Correlation of Circulating Peptide Levels with Plaques

A murine model for Alzheimer's disease, APP V717F transgenic mice, was used in this assay. These mice are described by Games, D., et al., *Nature* (1995) 373:523-527; Bales, K. R., et al., *Nature Genet.* (1997) 17:263-264; and by Holtzman, D. M., et al., *Proc. Natl. Acad. Sci. U.S.A.* (2000) 97:2892-2897. In this model, a mutant form of the human APP gene is expressed and results in an early onset form of familial Alzheimer's disease. Although the brains of these mice appear normal initially, Aβ deposition in the form of diffuse and neuritic plaques occurs at 6-15 months, although mice homozygous for the transgene show variability in that at 9-14 months of age, some mice develop Aβ deposits while others do not.

53 homozygous mice at 12 months were used in this study.

Plasma levels of $A\beta_{40}$, $A\beta_{42}$, and $A\beta_{40}/A\beta_{42}$ ratios were measured by ELISA in the plasma of these mice prior to administration of 500 μg of m266 and at various time intervals up to 24 hours after administering this antibody. After 24 hours, the mice were sacrificed, and the amount of Aβ deposition in the brain was assessed in the hippocampus and cortex as described by DeMattos, et al. *Proc. Nat'l.*

Acad. Sci USA (2001) 98:8850-8855, and evaluated as a percentage of brain covered by Aβ deposits.

As shown in FIGS. **1** A, B and C, if the percentage Aβ coverage due to deposition in the hippocampus is plotted on the x-axis against the levels of the peptides and their ratio in plasma on the y-axis prior to administration of the antibody, no correlation is found. Regardless of whether the percent Aβ deposition was essentially zero (0) or over 75%, the average level of $A\beta_{40}$ was approximately 250 (pg/ml) and of $A\beta_{42}$ approximately 400 (pg/ml). The ratio of $A\beta_{40}$ to $A\beta_{42}$ was thus approximately 0.5-0.6.

As shown in FIGS. **2** A and B, however, the plasma level of $A\beta_{40}$ strongly correlated with the percentage of Aβ deposition in hippocampus one hour after m266 injection, as did the ratio of $A\beta_{40}$ to $A\beta_{42}$.

FIGS. **3** A, B and C show similar results obtained 24 hours post injection. The levels obtained of $A\beta_{40}$ and the $A\beta_{40}$/$A\beta_{42}$ ratio strongly correlated with the % Aβ deposition in hippocampus The $A\beta_{42}$ levels also correlated with % Aβ deposition but not as well as $A\beta_{40}$ levels.

FIGS. **4** A, B and C show analogous results with respect to entry rate of the two Aβ peptides into the plasma and the calculated values for the entry rate as a function of the ratio of these peptides. The best correlations with Aβ deposition were rate of $A\beta_{40}$ entry and the ratio of $A\beta_{40}/A\beta_{42}$.

FIGS. **5** A and B show an alternate presentation of the data for plasma levels of $A\beta_{40}$ 24 hours and 1 hour after m266 injection. When the mice were grouped according to low, medium, or high Aβ coverage in the hippocampus, the animals with low Aβ deposition could be completely distinguished from those with high deposition as a function of the level of plasma $A\beta_{40}$.

Example 2

In a study similar to that set forth in Example 1, a cohort of 49 homozygous APP V717F mice were used. Before and after injection of 500 μg IV of m266, plasma samples were obtained at 5 minutes, 1 hour, 3 hours, 6 hours and 24 hours and levels of $A\beta_{40}$ and $A\beta_{42}$ were assessed as described in Example 1. The mice were sacrificed after 24 hours and 1 hemisphere was assessed for the percentage of the area of the hippocampus or cingulate cortex occupied by Aβ peptide (using quantitative Aβ immunofluorescence staining) and the area occupied by amyloid (by thioflavine-S (amyloid) staining). The regions from the other hemisphere were assessed for Aβ peptide by ELISA.

The Pearson correlation coefficient (Pearson r) and significance (P value) were determined between plasma Aβ values (pre and post injection of m266) and hippocampal Aβ or amyloid load using GraphPad Prism software (version 3.00 for Windows, San Diego, USA). Aβ load is defined as the percentage area of the hippocampus covered by Aβ-immunoreactive deposits. Amyloid load is defined as the percentage area of the hippocampus covered by thioflavine-S positive deposits. Correlations were also determined between the plasma Aβ accumulation over 24 hours (area under curve, AUC) and hippocampal Aβ load or amyloid load.

FIG. **6** shown the results obtained. Briefly, it was found that the base line levels (prior to injection) of $A\beta_{40}$, $A\beta_{42}$ and the calculated $A\beta_{40/42}$ ratio prior to injection with m266 did not correlate with percentage Aβ or amyloid deposition. However, following administration of m266, there were significant correlations between plasma $A\beta_{40}$, $A\beta_{42}$, and $A\beta_{40/42}$ ratio with both Aβ and amyloid burden in the hippocampus and cingulate cortex.

Statistical analysis of the results permits accurate prediction of hippocampal Aβ load in these mice based on plasma $A\beta_{40}$ levels 24 hours following m266 injection.

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20 25 30

Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Xaa Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Ser Gln Ser
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Thr His Val Pro Trp Thr Phe Gly Xaa Gly Thr Xaa Xaa Glu Ile Lys
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Arg

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Xaa Leu Val
35 40 45

Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Xaa Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Xaa Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp Thr Ala Val Tyr Tyr Cys
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Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
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Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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Arg


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Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
        35                  40                  45

Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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            20                  25                  30

Asp Gly Asn Ala Tyr Leu His Trp Phe Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Arg Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ser Gln Ser
                85                  90                  95

Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105                 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
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Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
145                 150                 155                 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
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Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
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            20                  25                  30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
        35                  40                  45

Ala Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Thr Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            100                 105                 110

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
        115                 120                 125

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
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145                 150                 155                 160

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
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Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
            180                 185                 190

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
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        275                 280                 285

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
    290                 295                 300

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
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Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
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Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
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Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
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Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
    370                 375                 380

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
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Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
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<223> OTHER INFORMATION: Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (58)..(58)
<223> OTHER INFORMATION: Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (63)..(63)
<223> OTHER INFORMATION: Xaa at position 63 is Thr or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (75)..(75)
<223> OTHER INFORMATION: Xaa at position 75 is Ala, Ser, Val, or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (76)..(76)
<223> OTHER INFORMATION: Xaa at position 76 is Lys or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (89)..(89)
<223> OTHER INFORMATION: Xaa at position 89 is Glu or Asp
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (107)..(107)
<223> OTHER INFORMATION: Xaa at position 107 is Leu or Thr

<400> SEQUENCE: 14

Xaa Val Gln Leu Val Glu Xaa Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Xaa Leu Val
35 40 45

Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Xaa Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Xaa Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp Thr Ala Val Tyr Tyr Cys
85 90 95

-continued

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Xaa Val Thr Val Ser Ser
100 105 110

<210> SEQ ID NO 15
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: humanized antibody
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(112)
<223> OTHER INFORMATION: Deglycosylated Humanized Antibody Heavy Chain Variable Region
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (56)..(56)
<223> OTHER INFORMATION: Xaa at position 56 is any amino acid, provided that if Xaa at position 57 is neither Asp nor Pro and Xaa at position 58 is Ser or Thr, then Xaa at position 56 is not Asn
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (57)..(57)
<223> OTHER INFORMATION: Xaa at position 57 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (58)..(58)
<223> OTHER INFORMATION: Xaa at position 58 is any amino acid, provided that if Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr

<400> SEQUENCE: 15

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
35 40 45

Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

<210> SEQ ID NO 16
<211> LENGTH: 442
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Humanized antibody
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(442)
<223> OTHER INFORMATION: Humanized Antibody Heavy Chain
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (56)..(56)
<223> OTHER INFORMATION: Xaa at position 56 is any amino acid, provided that Xaa at position 57 is neither Asp nor Pro and Xaa at position 58 is Ser or Thr, then Xaa at position 56 is not Asn
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (57)..(57)
<223> OTHER INFORMATION: Xaa at position 57 is any amino acid, provided

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that Xaa at position 56 is Asn and Xaa at position 58 is Ser or Thr, then Xaa at position 57 is Asp or Pro

<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (58)..(58)
<223> OTHER INFORMATION: Xaa at position 58 is any amino acid, provided that Xaa at position 56 is Asn and Xaa at position 57 is neither Asp nor Pro, then Xaa at position 58 is neither Ser nor Thr

<400> SEQUENCE: 16

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr
20 25 30

Ser Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val
35 40 45

Ala Gln Ile Asn Ser Val Gly Xaa Xaa Xaa Tyr Tyr Pro Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
115 120 125

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
130 135 140

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
145 150 155 160

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
165 170 175

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
180 185 190

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
195 200 205

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
210 215 220

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
225 230 235 240

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
245 250 255

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
260 265 270

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
275 280 285

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
290 295 300

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
305 310 315 320

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
325 330 335

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
340 345 350

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
355 360 365

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Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
    370                 375                 380

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
385                 390                 395                 400

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
                405                 410                 415

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
            420                 425                 430

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440


<210> SEQ ID NO 17
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus sp.
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(16)
<223> OTHER INFORMATION: Light chain CDR1
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(16)
<223> OTHER INFORMATION: Light Chain CDR1

<400> SEQUENCE: 17

Arg Ser Ser Gln Ser Leu Val Tyr Ser Asp Gly Asn Ala Tyr Leu His
1               5                   10                  15


<210> SEQ ID NO 18
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus sp.
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(17)
<223> OTHER INFORMATION: Heavy chain CDR2

<400> SEQUENCE: 18

Gln Ile Asn Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Ser Val Lys
1               5                   10                  15

Gly
```

The invention claimed is:

1. A method for diagnosing preclinical or clinical Alzheimer's disease in a subject, comprising measuring in the blood of the subject the level of $A\beta_{40}$ or $A\beta_{42}$ at a time interval after administration of an antibody that comprises a light chain variable region of the following sequence:

(SEQ ID NO: 7)

```
Asp Xaa Val Met Thr Gln Xaa Pro Leu Ser Leu Pro Val
1               5                   10

Xaa Xaa Gly Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser
    15                  20                  25

Gln Ser Leu Xaa Tyr Ser Asp Gly Asn Ala Tyr Leu His
            30                  35

Trp Phe Leu Gln Lys Pro Gly Gln Ser Pro Xaa Leu Leu
40                  45                  50

Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp
        55                  60                  65
```

-continued

```
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
                70                  75

Lys Ile Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr
    80                  85                  90

Tyr Cys Ser Gln Ser Thr His Val Pro Trp Thr Phe Gly
            95                  100

Xaa Gly Thr Xaa Xaa Glu Ile Lys Arg
105                 110
```

wherein:

Xaa at position 2 is Val or Ile;

Xaa at position 7 is Ser or Thr;

Xaa at position 14 is Thr or Ser;

Xaa at position 15 is Leu or Pro;

Xaa at position 30 is Ile or Val;

Xaa at position 50 is Arg, Gln, or Lys;

Xaa at position 88 is Val or Leu;

Xaa at position 105 is Gln or Gly;

Xaa at position 108 is Lys or Arg; and

Xaa at position 109 is Val or Leu;

and a heavy chain variable region of the following sequence:

(SEQ ID NO: 8)

```
Xaa Val Gln Leu Val Glu Xaa Gly Gly Gly Leu Val Gln
1               5                   10

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
    15                  20                  25

Phe Thr Phe Ser Arg Tyr Ser Met Ser Trp Val Arg Gln
            30                  35

Ala Pro Gly Lys Gly Leu Xaa Leu Val Ala Gln Ile Asn
40                  45                  50

Ser Val Gly Asn Ser Thr Tyr Tyr Pro Asp Xaa Val Lys
    55                  60                  65

Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Xaa Asn Thr
                70                  75

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Xaa Asp Thr
    80                  85                  90

Ala Val Tyr Tyr Cys Ala Ser Gly Asp Tyr Trp Gly Gln
            95                  100

Gly Thr Xaa Val Thr Val Ser Ser;
105                 110
```

wherein:

Xaa at position 1 is Glu or Gln;

Xaa at position 7 is Ser or Leu;

Xaa at position 46 is Glu, Val, Asp, or Ser;

Xaa at position 63 is Thr or Ser;

Xaa at position 75 is Ala, Ser, Val, or Thr;

Xaa at position 76 is Lys or Arg;

Xaa at position 89 is Glu or Asp; and

Xaa at position 107 is Leu or Thr;

wherein an elevated level of $A\beta_{40}$ or $A\beta_{42}$ as compared to a preselected control value identifies the subject as having preclinical or clinical Alzheimer's disease.

2. The method of claim **1**, wherein the time interval is less than 1 week.

3. The method of claim **1**, wherein the time interval is less than or equal to 24 hours.

4. The method of claim **1**, wherein the time interval is less than or equal to 3 hours.

5. The method of claim **1**, wherein the subject is human and the diagnostic antibody is a humanized antibody or an epitope-binding fragment thereof.

6. The method of claim **5**, wherein the diagnostic antibody has a light chain variable region of the sequence given by SEQ ID NO:9 and a heavy chain variable region of the sequence given by SEQ ID NO:10.

7. The method of claim **5**, wherein the humanized antibody or epitope-binding fragment thereof comprises a light chain of the sequence given by SEQ ID NO:11 and a heavy chain of the sequence given by SEQ ID NO:12.

* * * * *